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APPLICATION NUMBER: *60/530,547*
FILING DATE: *December 17, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/42358*

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EL919126855US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Liyan	He	Chandler, Arizona
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Additional inventors are being named on the 0 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Single Molecule Detection Using Molecular Motors

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 13 ☐ CD(s), Number

☑ Drawing(s) *Number of Sheets* 2 ☑ Other (specify) List accession nos.

☐ Application Date Sheet. See 37 CFR 1.76

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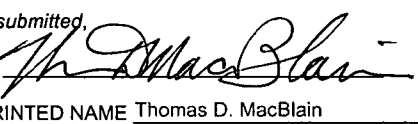
SIGNATURE 

TYPED or PRINTED NAME Thomas D. MacBlain

Date 12/17/03

REGISTRATION NO. 24,583 *(if appropriate)*

Docket Number: 9138-0134

TELEPHONE 602-530-8088

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1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
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2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

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Independent Claims		- 3** =		X		=	
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1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
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1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
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Other fee (specify) _____

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SUBMITTED BY				(Complete *(if applicable)*)	
Name (Print/Type)	Thomas D. MacBlain	*Registration No. (Attorney/Agent)*	24,583	*Telephone*	602-530-8088
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: He et al.

Filed: Herewith

Title: **SINGLE MOLECULE DETECTION USING MOLECULAR MOTORS**

CERTIFICATE OF MAILING BY EXPRESS MAIL
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1. Provisional Application for Patent Cover Sheet (1 page);
2. Fee Transmittal for FY 2004 (1 page in duplicate);
3. Specification (13 pages plus cover sheet);
4. Figures (2 pages);
5. List of accession numbers for relevant F_1-ATPase sequences (29 pages);
6. Check for $80.00; and
7. A return receipt postcard.

December 17, 2003
Date

Suzanne Shields
Suzanne Shields

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Express Mail Label No. EL919126855US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Provisional Patent Application

Title: **SINGLE MOLECULE DETECTION USING MOLECULAR MOTORS**

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SINGLE MOLECULE DETECTION USING MOLECULAR MOTORS

Financial assistance for this project was provided by the U.S. Government, DARPA # N66001-03-C-XXXX; thus the United States Government has certain rights to this invention.

Various DNA hybridization methods have been developed in attempts to detect single molecules of nucleic acid (Singh-Zochhi et al., 2003; Castro et al., 1995, 1997, 2000). These methods have shown great potential for ultra-sensitive detection of nucleic acid. However they inherited the intrinsic limitation of DNA hybridization assays such as nonspecific binding, hybridization kinetics, and the requirement for a purified sample. The present invention provides methods that overcome the above limitations, thereby yielding higher sensitivity and accuracy than other methods.

The present invention provides novel devices and methods for using such devices to detect a target nucleic acid sequence in a sample. The methods disclosed herein detect a DNA target as the result of ligation with two target-specific nucleic acids, which are ligated in the presence of the nucleic acid target. The thus-ligated product is used to bridge a molecular motor and a detection probe. The probe reveals the motion imparted by the molecular motor to the bridging ligated product that is indicative of the nucleic acid target. The motion imparted to the probe is observed by an appropriately chosen means of detection. The methods of the invention are capable of detecting single molecules of the target nucleic acid, and thus provide an extremely sensitive technique for target detection that is of wide applicability, including but not limited to clinical diagnostics, forensic analysis, gene expression analysis, and DNA sequencing.

Thus, in one aspect, the present invention provides methods for detecting a target nucleic acid comprising:

(a) providing first and second target-specific nucleic acids, wherein the first and second target-specific nucleic acids each comprise sequences complementary to the target nucleic acid; wherein the first target specific nucleic acid is bound to a first affinity tag and the second target-specific nucleic acid is bound to a second affinity tag, wherein the first affinity tag is capable of binding to a molecular motor, and wherein the second affinity tag is capable of binding to a detection probe;

(b) contacting the first and second target-specific nucleic acids to a sample under conditions whereby the first and second target-specific nucleic acids will hybridize to the target

nucleic acid if the target nucleic acid is present in the sample, wherein upon hybridization to the target nucleic acid the first and second target-specific nucleic acids are directly adjacent to each other;

(c) ligating the first and second target-specific nucleic acids together;

(d) binding the molecular motor to the first affinity tag and the detection probe to the second affinity tag;

(e) inducing movement of the molecular motor; and

(f) detecting movement of the molecular motor through the detection probe, wherein the movement of the molecular serves to detect the target nucleic acid in the sample.

The target nucleic acid can be any nucleic acid that can serve as a bridge between a molecular motor and a detection probe to detect motor-induced motion and for which the means of formation of that bridge is specific to that target nucleic acid. Thus, the target nucleic acid can comprise DNA or RNA and can be single stranded or double stranded. In a preferred embodiment, the target nucleic acid is double stranded. In a more preferred embodiment, the target nucleic acid is a double stranded DNA.

. The first and second target specific nucleic acids can be any pair of nucleic acid sequences that are both complementary to the same target nucleic acid., so long as the first and second target specific nucleic acids are capable of hybridizing to adjacent segments of a target sequence so that the 3' end of the first target-specific nucleic acid is directly adjacent to the 5' end of the second target-specific nucleic acid. There is no other specific nucleic acid sequence requirement for the first and second target specific nucleic acids.)- The first and second target specific nucleic acids can independently comprise DNA or RNA and can be single stranded or double stranded. In a preferred embodiment, the first and second target specific nucleic acids are both double stranded. In a more preferred embodiment, the first and second target specific nucleic acids are both double stranded DNA.

As used herein the term "directly adjacent" means juxtaposed 5' phosphate and 3' hydroxyl termini of two adjacent target-specific nucleic acids hybridized to the complementary target nucleic acid, which can be ligated together by the action of a nucleic acid ligase.

The first affinity tag and the second affinity tag may be the same or different as is most suitable for their ultimate attachment to the specific molecular motor and the detection probe employed. Preferably, the first and second affinity tags are different.

The first affinity tag can bind to the molecular motor and the second affinity tag can bind to the detection probe either directly (for example by a covalent bond between the target-specific nucleic acid and the affinity tag) or indirectly through another molecule. In a preferred embodiment, the first and second affinity tags bind indirectly to the molecular motor and the detection probe, respectively. In this preferred embodiment, the affinity tag binds directly to the target-specific nucleic acid sequence and to an affinity target, wherein the affinity target is bound to the molecular motor or the detection probe. Together, an affinity tag and affinity target make up a binding pair. Either member of a binding pair can be used as an affinity tag and either member can be used as an affinity target. An affinity target includes both separate molecules and portions of molecules, such as an epitope of a protein that interacts specifically with an affinity tag. Antibodies, either member of a receptor/ligand pair, and other molecules with specific binding affinities can be used as affinity tags. Binding an affinity tag to the target-specific nucleic acids thus permits an indirect linkage between the target-specific nucleic acids and the molecular motor or the detection probe. An affinity tag that interacts specifically with a particular affinity target is said to be specific for that affinity target. For example, an affinity tag which is an antibody that binds to a particular antigen is said to be specific for that antigen. Complementary nucleotide sequences can be used as binding pairs.

A non-limiting example of a binding pair is biotin/avidin. Other non-limiting binding pair examples include digoxigenin (DIG)/ anti-digoxigenin antibody and other antigen/antibody pairs. Epitope tags, such as a his-tag, and antibodies directed against the epitope tag (or fragments thereof) are further examples of binding pairs for use with the methods of the present invention. Those of skill in the art will understand that certain embodiments listed herein as indirect binding of the affinity tag and the molecular motor or detection probe can also be used for direct binding embodiments. For example, where the second affinity tag is an epitope tag as described above, the detection probe can be a labeled antibody against the epitope tag. Many further such examples will be readily apparent to those of skill in the art.

The affinity tags are bound to the first and second target-specific nucleic acids so as to not interfere with the ability of the first and second target-specific nucleic acids to be ligated together after hybridization to the target nucleic acid. In a preferred embodiment, one of the affinity tags is bound at or near the 5' end of one of the target specific nucleic acid sequences, and the other affinity tag is bound at or near the 3' end of the other target-specific nucleic acid

sequence, so as to permit juxtaposition of the 5' phosphate and 3' hydroxyl termini of the two adjacent target-specific nucleic acids at the desired site of ligation after hybridization of the target-specific nucleic acids to the target nucleic acid. Such design of the target-specific nucleic acids and the affinity tags is well within the level of skill of those in the art.

The sample in which detection of the target nucleic acid is to be assessed can be any sample of interest, including but not limited to genomic DNA, cell lysates, tissue homogenates, forensic samples, environmental samples, and isolated nucleic acid samples from cells, tissues, or complete organisms.

Optimization of conditions for contacting the first and second target-specific nucleic acids to a sample under conditions whereby the first and second target-specific nucleic acids will hybridize to the target nucleic acid if the target nucleic acid is present in the sample can be readily accomplished by those of skill in the art. The hybridization conditions are thus optimized to limit hybridization/ligation to those situations where the target nucleic acid is present. Such optimization includes consideration of the nucleic acid probe sequence and length, reaction buffer, reaction temperature, and reaction time.

The ligation step of the methods of the invention can be accomplished by techniques known to those of skill in the art using commercially available nucleic acid ligases. Any DNA ligase is suitable for use in the disclosed method. Preferred ligases are those that preferentially form phosphodiester bonds at nicks in double-stranded DNA. That is, ligases that fail to ligate the free ends of single-stranded DNA at a significant rate are preferred. Thermostable ligases are especially preferred. Many suitable ligases are known, such as T4 DNA ligase (Davis et al., Advanced Bacterial Genetics--A Manual for Genetic Engineering (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1980)), E. coli DNA ligase (Panasnko et al., J Biol. Chem. 253:4590-4592 (1978)), AMPLIGASE.RTM. (Kalin et al., Mutat Res., 283(2):119-123 (1992); Winn-Deen et al., Mol Cell Probes (England) 7(3):179-186 (1993)), Taq DNA ligase (Barany, Proc. Natl. Acad Sci. USA 88:189-193 (1991), Thermus thermophilus DNA ligase (Abbott Laboratories), Thermus scotoductus DNA ligase and Rhodothermus marinus DNA ligase (Thorbjarnardottir et al., Gene 151:177-180 (1995)). T4 DNA ligase is preferred for ligations involving RNA target sequences due to its ability to ligate DNA ends involved in DNA:RNA hybrids (Hsuih et al., Quantitative detection of HCV RNA using novel ligation-

dependent polymerase chain reaction, American Association for the Study of Liver Diseases (Chicago, IL, Nov. 3-7, 1995)).

In a most preferred embodiment, ligation is accomplished by use of a ligation chain reaction. The term "ligation chain reaction" ("LCR") describes the process pioneered by Landegren et al. (1988 Science 241, 1077-1080). This process detects the presence of given DNA sequences based on the ability of two probes to anneal directly adjacent to each other on a complementary target DNA molecule. The two probes are then joined covalently by the action of a DNA ligase, provided that the nucleotides at the junction are correctly base-paired. Thus single nucleotide substitutions can be distinguished. This strategy permits the rapid and standardized identification of single-copy gene sequences in genomic DNA. Furthermore, due to its high specificity, LCR can be performed in crude samples, without the need for purifying the nucleic acid target, which significantly simplifies the assay process.

The disclosed method detects target nucleic acid through ligation chain reaction (Cheng et al., 1996) to generate a DNA sequence that has affinity tags on both ends so that it can serve as a bridge between the molecular motor and the detection probe. Ligation chain reaction requires the formation of juxtaposed 5' phosphate and 3' hydroxyl termini of the two adjacent target-specific nucleic acids, which are hybridized to a complementary nucleic acid target. The ligation will occur only if the target-specific nucleic acids are perfectly paired to the target nucleic acid and have no gap between them. Therefore, this method is much more specific than hybridization alone and a single-base substitution can be easily detected. In the disclosed methods using double stranded target-specific nucleic acids and target nucleic acid, thousands of copies of the ligated products can be generated from a single copy of target nucleic acid. LCR does not actually copy the target itself. However, after the first pair of target-specific nucleic acids is ligated, double stranded DNA products can be denatured at higher temperature to allow another pair of target-specific nucleic acid probes to hybridize to the same target, followed by further ligation. The process is similar to PCR except the target molecules are not being copied. In this embodiment, thermal cycling is most preferred. In a non-limiting example, conditions employed include an initial hybridization step at 95°C for two minutes, followed by 19 cycles of 1 minute at 95°C and 4 minutes at 65°C in the presence of a thermostable DNA ligase and appropriate reaction components. Those of skill in the art are well-versed in modifying such cycling

conditions to provide optimal hybridization based on the use of different nucleic acid sequences or different buffer conditions.

The molecular motors of the invention include any biological or synthetic molecule capable of induced translational or rotational movements that are capable of detection. In a preferred embodiment, the molecular motor is a biomolecular motor. Non-limiting examples of such biomolecular motors are F_1-ATPases (see, for example, the attached list of accession number for relevant F_1-ATPase sequences), actomyosin, ciliary axonemes, bacteria flagellar motors, kinesin/microtubules, and nucleic acid helicases and polymerases. In some cases the molecular motor may need to be immobilized (i.e. secured in place) for detection. For example, it may be necessary to immobilize the molecular motor if the rotation needs to be visualized or if the detection depends on the perturbation of the local environment such as micro current or impendence. A series of molecular motors can be immobilized on a surface to generate a molecular motor array. If each motor is coated with different affinity targets and different first target-specific nucleic acids (specific to the same or different target nucleic acids) are labeled with different affinity tags, this molecular motor array can be used to detect multiple target nucleic acids in such manner similar to a gene chip. As used herein, an "array" comprises a solid surface, with molecular motors attached to said surface. Arrays typically comprise a plurality of molecular motor linked to different capture groups that are coupled to a surface of a substrate in different, known locations. For example, there are several silane derivatives to attach a variety of functional groups to a glass surface. The term "solid surface" as used herein refers to a material having a rigid or semi-rigid surface. Such materials will preferably take the form of chips, plates, slides, cover slips, small beads, pellets, disks or other convenient forms, although other forms may be used. The surfaces are generally coated with an affinity target.

In a preferred embodiment, the first affinity tag attaches to a moiety on the moving component of the motor. By way of example, the rotating subunits on the F1-ATPase include the γ and ε subunits, while the α, β, and δ subunits do not rotate. The γ subunit is currently strongly preferred because the ε subunit acts as an ATPase inhibitor Thus, where the F1-ATPase is used as the molecular motor, it is preferred that the first affinity tag bind (directly or indirectly) to the γ subunit, while the molecular motor is attached to a substrate through the α, β, or δ subunits. The α or β subunits are currently preferred because they provide a three-point attachment due to the stoichiometry of these subunits in the protein. For example, the

"moiety" may be a cysteine residue created by site-directed mutagenesis at a specific position of a protein-based biomolecular motor, such as the γ subunit of F1-ATPase. The first affinity tag can be attached to the cysteine residue through linkage to its sulfhydryl group. Alternatively, an affinity target can be used to coat the molecular motor, and can interact with the affinity tag. This molecular motor coated with affinity targets can then be linked specifically to the affinity tag on the first target-specific nucleic acid. As will be apparent to one of skill in the art, site directed mutagenesis can be used to introduce a cysteine residue (or other useful residues) to various protein-based biomolecular motors so that they can be linked to affinity tags. Furthermore, there are a variety of covalent modification reagents that can modify specific amino acid side chains.

The detection probe can be anything that is capable of attaching to the affinity tag on the second target-specific nucleic acid and providing a means of detecting the movement generated by the molecular motor, such as metallic nanoparticles (rods, spheres, quantum dots, etc.) fluorescent dyes, and nanoparticles labeled with fluorescent dyes. In a non-limiting example, gold rod detection probes capable of visual observation by microscope are attached to the second target-specific nucleic acid through a biotin bond. In a non-limiting embodiment, the gold nanorod is coated with anti-DIG antibody (the affinity target), which binds specifically to a DIG (Digoxigenin) second affinity tag. In another preferred embodiment, the gold nanorods can directly link to alkanethio modified oligonucleotides.

Inducing movement of the molecular motor is done by standard methods in the art for a given molecular motor. For example, the movement of F1 ATPases is induced by adding ATP (Noji, H., Yasuda, R., Yoshida, M. and Kinosita, K. (1997) Nature 386, 299-302). Those of skill in the art are able to determine how to induce movement of other known molecular motors using similar published protocols. The only motion that will be detected will result from molecular motors that are connected to the detection probe. Since that will depend upon the presence of the nucleic acid bridge resulting from specific hybridization of the target specific nucleic acids and the target nucleic acid (and the subsequent ligation), observation of this motion will identify the presence of the target nucleic acid.

Detecting movement of the molecular motor through the detection probe can be accomplished by any suitable means. In one embodiment, direct visualization of the movement is used. In a non-limiting example, gold rod detection probes capable of visual observation by

microscope are attached to the second target-specific nucleic acid through a biotin/avidin interaction. Other means of observation include, but are not limited to single molecule fluorescence resonance energy transfer, fluorescence lifetime anisotropy, and atomic force microscopy. Beside microscopy, other methods can be used to observe the rotation of detection probe, including but not limited to: (1) attaching the molecular motor onto a nano-electrode and measure the micro current change or impendence change produced by rotation; (2) Measuring single molecule anisotropy; (3) Using a surface plasmon resonance biosensor to measure the surface plasmon resonance change during metallic nanorods rotation; and (4) Attaching a fluorescent label such as Pacific Blue (Molecular Probes) on non-rotating part of the molecular motor. Rotation can be observed through periodic quenching of the fluorescence signal by a quencher detection probe attached to the rotating part of the molecular motor. Alternatively, the fluorescent label can be attached to the rotating part of the molecular motor and the quencher can be linked to the non-rotating part. .

Since the methods of the invention are capable of detecting single molecules of a target, they provide a precise means to quantify the amount of target present. In one embodiment, the number of rotating molecules is determined by visualization and a calculation is made for the fraction of the total sample that is being viewed. This is not possible with fluorescent detection methods in current use with DNA microarrays.

Example 1

As shown in Fig. 1, one embodiment of the disclosed methods comprises the following steps:

1. As indicated in Fig. 1a, a first affinity tag is attached to the 5' end of the first target-specific DNA strand. A second affinity tag is attached to the 3' end of second target-specific DNA strand. The second target-specific DNA strand is phosphorylated either during chemical synthesis, or after synthesis using a polynucleotide kinase (Fig. 1a).

2. In Fig. 1b the first and second target-specific nucleic acid strands are hybridized to the target nucleic acid so that the 3' end of the first target-specific strand is directly adjacent to the 5' end of the second target-specific strand.

3. In Fig. 1c the first and second target-specific DNA strands are ligated, to generate a double-stranded DNA sequence in which one of the strands contains the first and the second affinity tags at the 5' and 3' ends, respectively.

4. In Fig. 1d the double-stranded DNA that contains the affinity tags is then used as a bridge between a molecular motor and the detection probe used to detect the motion generated from the motor via the affinity tags. The first affinity tag attaches specifically to a moiety on a moving component of the molecular motor while the second affinity tag is specific to the detection probe. In the specific preferred and exemplary embodiment the first affinity tag, a biotin, binds to an avidin attached to the rotating part of the motor and therefore links the motor to the first target-specific nucleic acid. The motor shown is the F_1-ATPase biomolecular motor. In the specific example, the detection probe comprises gold nanorods that can be visualized by microscopy, attached to the second target-specific nucleic acid through interactions between digoxigenin (DIG) and anti-digoxigenin antibody. The particular gold rods are of such sizes that they efficiently scatter red and green light from their long and short axes, respectively, such that when viewed through a polarizing filter with dark-field microscopy, the rotating rods appear as alternating red and green spots.

5. Immobilization occurs after assembly of the components in step 4 (Fig. 2a). Immobilization is effected by histadine binding of the nonrotational F1 motor structure to a nickel surface.

6. The movement of the molecular motor is induced by adding ATP (Fig. 2b). The only motion that is detected results from motors that are connected to the attached detection probe. Since that depends upon the presence of the double-stranded DNA bridge and because the double stranded DNA only results from the specific hybridization to the target DNA, observation of this motion identifies the presence of the target DNA.

Example 2

The following are two examples for detecting multiple target nucleic acids simultaneously:

Approach 1: Detection probes comprising gold rods of different sizes are used to detect ligated DNAs that are specific to pBR322 and Lambda DNA simultaneously using the following procedures:

1. Gold nanorods of different lengths are prepared because the length determines the wavelength of light scattered from it. The short and long gold rods are prepared to enable them to bind to digoxigenin (DIG) and dinitrophenyl-X (DNP-X), respectively.
2. Two pair of target-specific nucleic acids are prepared that are designed to hybridize specifically with pBR322 and Lambda DNA, respectively.
3. To enable the target-specific nucleic acid pairs to bridge between F_1 ATPase and a gold nanorod, one target-specific nucleic acid from each pair is labeled with biotin while the other target-specific nucleic acid is labeled with DIG and DNP-X for pBR322 and Lambda DNA, respectively.
4. LCR is performed with the two target-specific nucleic acid pairs.
5. The ligated products are linked to avidin-coated F_1 ATPase though biotin/avidin binding.
6. The assembly is completed upon attachment of the gold nanorods of different lengths.
7. Observation of ATP-dependent rotation of different colored nanorods indicates the presence of the corresponding target.

Approach 2: Ligated DNAs that are specific to pBR322 and Lambda DNA are detected simultaneously on an antibody-coated-F_1 ATPase array using the following procedures:

1. F_1-ATPase is prepared to enable binding to either anti-DIG antibody and anti-DNP antibody, respectively.
2. Anti-DIG-coated F_1 ATPase and anti-DNP-coated F_1 ATPase are immobilized on different locations on a cover slip.
3. Two pairs of target-specific nucleic acids are prepared that are designed to hybridize specifically with pBR322 and Lambda DNA, respectively.
4. To enable the target-specific nucleic acid pairs to bridge between F_1 ATPase and a gold nanorod, one target-specific nucleic acid of each pair is labeled with biotin while the other target-specific nucleic acid is labeled with DIG and DNP-X for pBR322 and Lambda DNA, respectively.
5. LCR is performed with the two target-specific nucleic acid pairs.

6. The ligated products from pBR322 and Lambda DNA are linked to anti-DIG-coated F_1 ATPase anti-DNP-coated F_1 ATPase, respectively.

7. The assembly is completed upon attachment of the avidin coated gold nanorods.

8. Observation of ATP-dependent rotation at different location on the cover slip indicates the presence of the corresponding target nucleic acid.

In another aspect, the present invention provides kits for nucleic acid detection comprising first and second target-specific nucleic acids, wherein the first and second target-specific nucleic acids each comprise sequences complementary to a target nucleic acid, wherein upon hybridization to the target nucleic acid the first and second target-specific nucleic acids will be directly adjacent to each other; wherein the first target specific nucleic acid is bound to a first affinity tag and the second target-specific nucleic acid is bound to a second affinity tag, wherein the first affinity tag is capable of binding to a molecular motor, and wherein the second affinity tag is capable of binding to a detection probe.

In preferred embodiments, the kit further contains a molecular motor that binds to the first affinity tag and/or a detection probe that binds to the second affinity tag. In a further embodiment, the molecular motor is bound to a solid support, such as a glass coverslip or other suitable support. The support can be derivatized in any manner suitable for binding to the molecular motor.

The present invention also provides a composition comprising first and second target-specific nucleic acids base-paired with a target nucleic acid and directly adjacent to each other, wherein the first target specific nucleic acid is bound to a first affinity tag capable of binding to a molecular motor and the second target-specific nucleic acid is bound to a second affinity tag capable of binding to a detection probe. In a further embodiment, the first and second target-specific nucleic acid sequences are ligated together.

The present invention also provides a composition comprising:

(a) a nucleic acid complex comprising a first target-specific nucleic acid bound to a first affinity tag capable of binding to a molecular motor; a second target-specific nucleic acid bound to a second affinity tag capable of binding to a detection probe, and a target nucleic acid, wherein the first and second target-specific nucleic acids are base-paired with the target nucleic acid and ligated together;

(b) a molecular motor bound to the first affinity tag; and

(c) a detection probe bound to the second affinity tag.

The present invention further provides a composition comprising:

(a) a solid support; and

(b) a plurality of molecular motors attached to the solid support, wherein the plurality of molecular motors comprise an affinity target for binding to a specific affinity tag.

In a preferred embodiment, the plurality of molecular motors comprises more than one type of molecular motor. In a further preferred embodiment, the different types of molecular motors on the support comprise different affinity targets that are specific for different affinity tags. In a further preferred embodiment, the composition further comprises a first target-specific nucleic acid bound to a first affinity tag that binds to the affinity target on the molecular motor. In a further preferred embodiment, the first target specific nucleic acid is hybridized to a target nucleic acid, and the target nucleic acid is further hybridized to a second target-specific nucleic acid that is bound to a second affinity tag, wherein the second affinity tag is bound to a detection probe.

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Figure 1

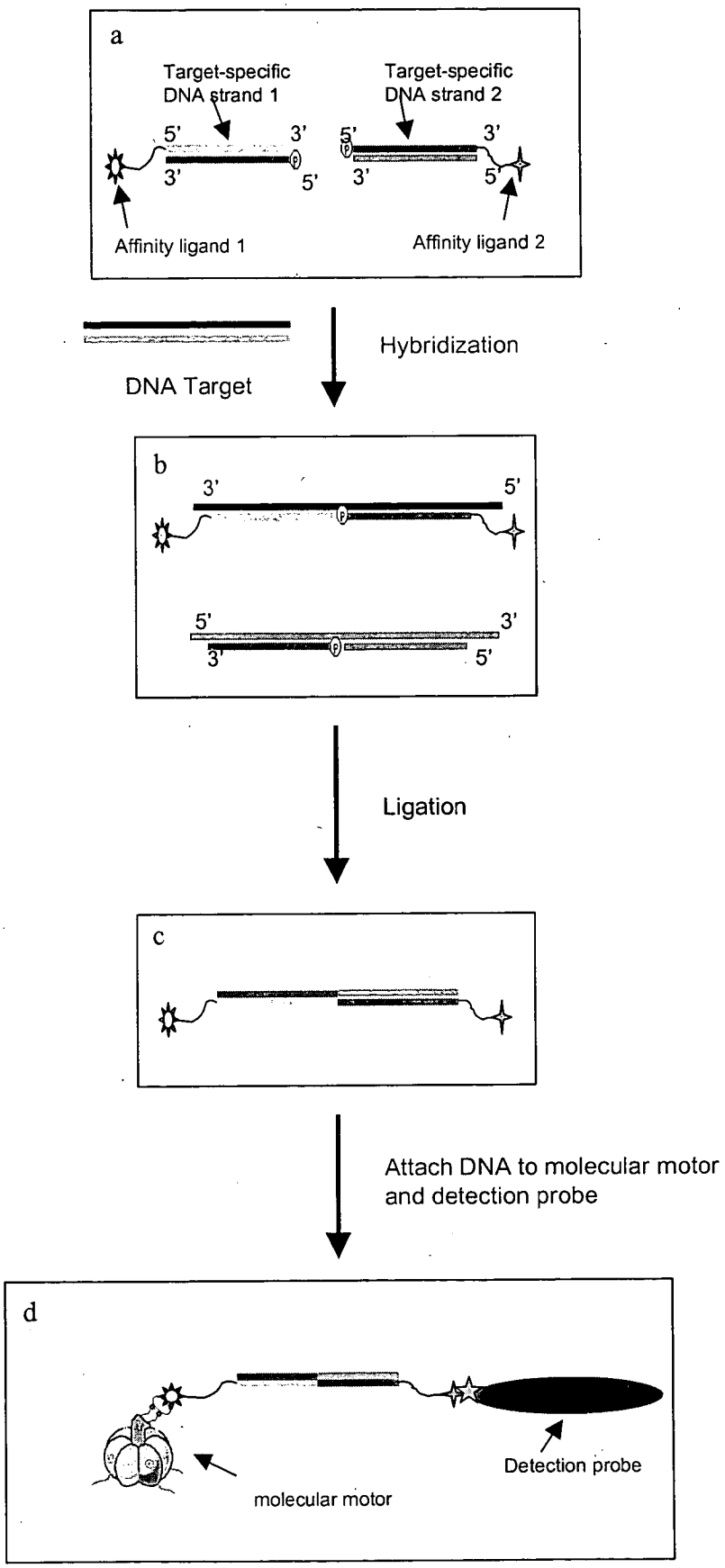
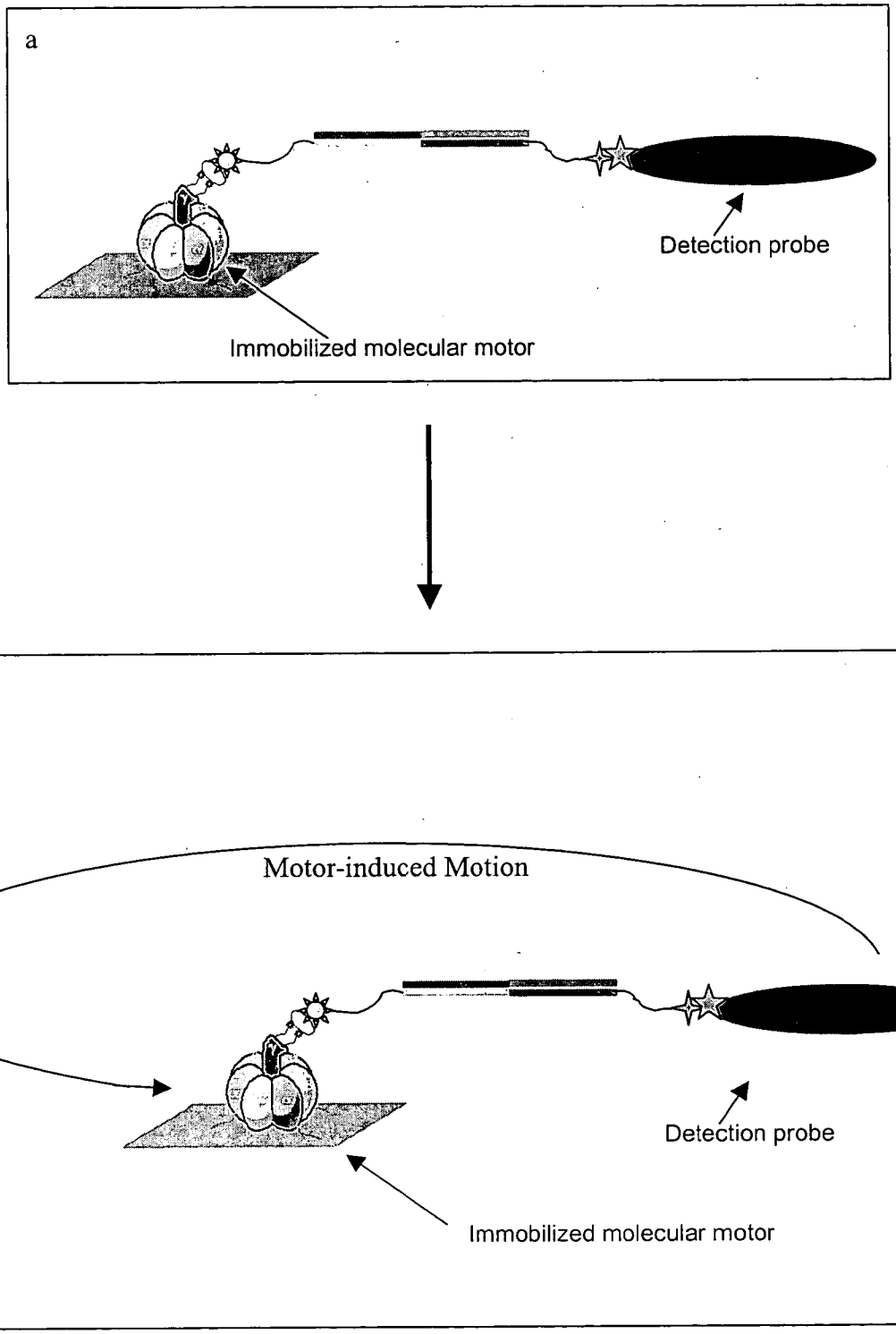

a
Target-specific DNA strand 1
Target-specific DNA strand 2
5'
3'
3'
5'
5'
3'
3'
5'
Affinity ligand 1
Affinity ligand 2
DNA Target
Hybridization
b
3'
5'
5'
3'
3'
5'
Ligation
c
Attach DNA to molecular motor and detection probe
d
Detection probe
molecular motor

Figure 2


a
Detection probe
Immobilized molecular motor
b
Motor-induced Motion
Detection probe
Immobilized molecular motor